Message

From: Schlosser, Paul [Schlosser.Paul@epa.gov]
Sent: 4/24/2014 4:04:53 PM
To: Mel Andersen [MAndersen@thehamner.org]; Subramaniam, Ravi [Subramaniam.Ravi@epa.gov]
Subject: RE: Formaldehyde Workshop: Session 1 Planning Call

I believe Ravi asked me to send that out of concern that the session wouldn't address the points and questions on which EPA needs input. Originally I had not intended to send anything, since I am just a discussant. But I thought it could represent one view, and as I said in the email, something that the presenters might consider. One of the options I said was for their data to refute the analysis.

I am aware that over 99% of formaldehyde in simple aqueous systems will be in the form of the acetal, but then that's why I put "free" in quotes. Since DPX formation rate constant that comes from Henry Heck's in vitro studies must reflect this degree of hydration, that it is the effective substrate for those reactions. My "musings" are really just a specific quantification of what Henry Heck concluded long ago. Even after you account for hydration, there must be some other mechanism by which the cell and DNA are protected from the majority of the measurable (~ 400 uM) endogenous level.

I am sorry that I am not familiar with your 2010 paper, but I was only half-joking when I said that I've been trying to keep away from formaldehyde for some time now. However I think the dG data that I analyzed was more recent than that, and I have the older Conolly code on my machine.

The primary goal of the analysis was to estimate steady-state or long-term dG levels from the 6-hr data that was available from Swenberg last year, to allow a more direct comparison of that with the endogenous levels, which are presumably at steady state and represent relative risk.

What follows is ***not*** something I plan to present or discuss next week, but FYI. I think the suggestion that I give higher relevance to Harvey's SRA talk, which is not peer-reviewed/published, over my own analysis is not really fair.

We had a chance to review the Schroeter et al. paper prior to publication and provided comments (via Rory) at that time. If he didn't forward that to you, that's unfortunate. From my review of the published version, none of the major points raised in my comments were addressed.

I'll note that this paper too does not distinguish between free and bound HCHO, and in particular it assumes that all of the endogenous formaldehyde is free miscible with exogenous. This must substantially change the prediction of tissue dose vs. exposure, but the parameters for HCHO removal and formation of DPX are the same ones as the Conolly et al. (2000) paper which completely ignores endogenous HCHO. The ability of the revised model to still match the observed DPX data was not shown.

Below is a plot from that paper. For 0 ppm exposures it predicts that the endogenous level drops to zero as one moves from the "blood" layer (at 375 um) to the air-tissue interface. This is clearly unrealistic. Endogenous formaldehyde would be produced throughout the tissue, and blood capillaries that deliver it from the rest of the body don't exist at a single depth into the tissue. I doubt that endogenous levels have that kind of gradient.

The improvement in the CFD mesh and the linking of the tissue phase of the model with the air phase are clear improvements in the modeling over what was done back in the '90s. In that regard this is a major advancement. But the assumption that endogenous formaldehyde comes from a 'point source' in the tissue stack is not biologically realistic and the fact that the model parameters/predictions were never re-evaluated against the DPX data means that at a minimum we'd have to test that before using the model. What's shown below for 1 ppm is roughly a tissue average concentration of 0.3 mM. Using Heck's DPX formation rate and the published DPX clearance rate that I'd used previously, this leads to a predicted level of ~ 140 pmol DPX/mg DNA after a 6 hr exposure. While that prediction (shown below) is for humans, the fact that this is 2 orders of magnitude higher than what was observed in the rat at 1 ppm indicates a serious problem with the model.

I'm not sure what all you are considering as you continue to revise your models and analyses. But I can find and forward a copy of what I wrote up for the draft of Jeff's paper, if you want to understand in more detail why I would not recommend use of it as is.

-Paul

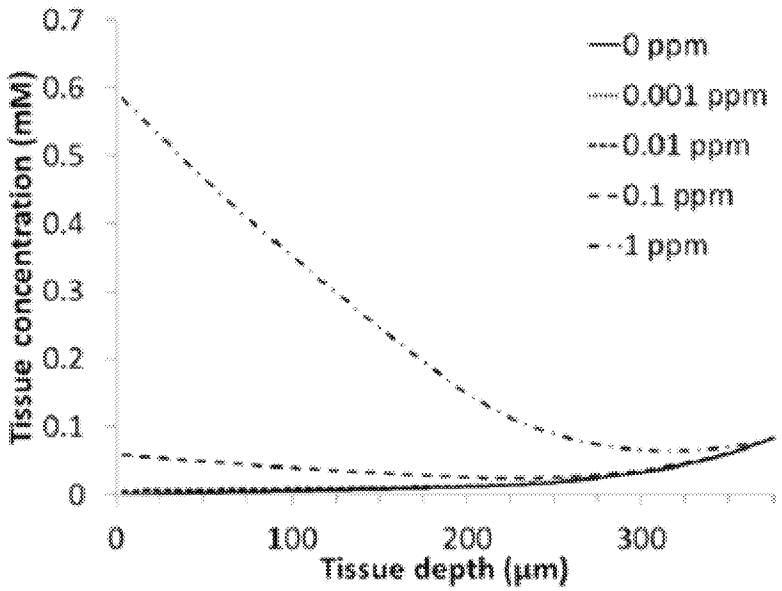

FIG. 4. Predicted formaldehyde nasal tissue concentrations in the human

From: Mel Andersen [mailto:MAndersen@thehamner.org]
Sent: Thursday, April 24, 2014 10:12 AM
To: Schlosser, Paul; Subramaniam, Ravi
Subject: RE: Formaldehyde Workshop: Session 1 Planning Call

Paul/Ravi,

I found this e-mail somewhat irritating. You seem to be moving away from the purported rationale for the meeting to a position where you want the panel to weigh in on assessing their comfort with your unpublished musings about formaldehyde.

As you know, The Hamner has been involved in developing PK models that account for exogenous and endogenous input of formaldehyde for the past 5 years. Some initial work appeared in our 2010 paper trying to link gene expression, toxicity and preliminary PK structures for both inhaled formaldehyde and endogenous production to predict protein cross-links (Andersen et al., Toxicol. Sci., 2010). With Jerry Campbell taking the lead, we continued the PK modeling work using newer adduct data from Jim's papers and have reported these results at the SRA meeting last year (Clewell et al, (2013), "Pharmacokinetics of Inhaled Formaldehyde and the Impact of Endogenous Levels"). We have delayed publication of the our recent PK modeling work pending availability of more complete data sets coming from Jim Swenberg's team. In addition, we have worked to develop CFD models that account for endogenous formaldehyde in a paper in press from Jeff Schroeter et al., Toxicol. Sci., 138, 412-424, 2014. As the development of these model structures move forward, we are refining ideas about pools of formaldehyde and cellular compartmentalization – especially in relation to cytosolic and nuclear compartments.

We will be completing our PK modeling work in the next few months. The published work will be available for general review and discussion by US EPA and by others. Nonetheless, we hope it will be used in EPA deliberations about endogenous formaldehyde, but EPA needs it in an appropriate form to assess model structure and performance and to consider if you agree with description of formaldehyde in tissues. I would be pleased to have a conversation about key ideas – most tissue formaldehyde is reversibly bound with various nucleophiles, the reactivity in tissue is likely due to reactions with the formaldehyde acetal and displacement of water by other nucleophiles, the early formaldehyde

assays assessed levels of loosely bound formaldehyde that can be reacted irreversibly to form hydrazone derivatives. The relevant concentration in specific compartments is more likely to be this pool of "bound", but available formaldehyde rather than free CH_2O. My experience as a chemist and biochemist strongly support this model structure.

My point in this longish e-mail is a question about the meeting itself. What is it that you want covered in these sessions? Published papers with specific information, work in progress that has been reported, but not yet finalized, or comment on some set of unpublished calculations?

I am interested in your response to this e-mail and your opinions about whether you should also send some of this material to the presenters and discussants? I actually don't think any of this should be going to the panel members and discussants at this late stage. However, I feel that your distribution of the unpublished musings biases the panel inappropriately.

Mel

Melvin E. Andersen, PhD, CIH, DABT, FATS
Chief Science Officer &
Director, Institute of Chemical Safety Sciences
The Hamner Institutes for Health Sciences
Six Davis Drive PO Box 12137
Research Triangle Park, NC 27709-2137
Tel: 919-558-1205 Fax: 919-558-1300
e-mail: MAndersen@thehamner.org

From: Schlosser, Paul [mailto:Schlosser.Paul@epa.gov]
Sent: Thursday, April 24, 2014 9:15 AM
To: Wignall, Jessica; Subramaniam, Ravi; Mel Andersen; Edna Mangum; Appling, Dean; Lisa Peterson; Ross, Jeff; Martyn Smith; James Swenberg
Cc: kim.osborn@icfi.com; Malloy, Maureen; Sharp, Codi
Subject: RE: Formaldehyde Workshop: Session 1 Planning Call

Colleagues,

Ravi recalled that I'd done an analysis based on some of Dr. Swenberg's data in combination with the formaldehyde inhalation dosimetry model of Conolly et al. (2000) to contrast the levels of N^2-hydroxymethyl-deoxyguanine (dG) adducts with what's known or can be estimated for endogenous vs. exogenous levels, and asked that I send it around. I've attached the piece. It's just over 2 pages, though a bit dense.

Part of this may be trumped by recent data that Jim mentioned on the phone.

In short the Conolly et al. model used observed DNA-protein-crosslink (DPX) data and a rate constant for DPX formation from in vitro experiments to effectively estimate the nasal tissue levels of HCHO at various exposure levels. I then extended the model to predict dG formation and clearance (assuming formation is proportional to HCHO levels and clearance is first-order), calibrating the extended model to Jim's dG data from 6-h exposures. I then used the model to predict what dG levels would be given continuous HCHO exposure or a long-tern 5 d/wk, 6 h/d pattern.

Also, I can use the model to back calculate what level of "free" HCHO must be in the cells to be consistent with the observed endogenous dG levels. As stated, reported/measured endogenous formaldehyde levels are ~ 400 uM, but if that formaldehyde was as available to form dG as the exogenous formaldehyde in Jim's experiments, then the endogenous dG levels should be ~ 40 times higher than observed. Put another way, the endogenous dG levels, based on this modeling, are only consistent with a "free" endogenous HCHO level of 10.4 uM, not 400 uM. This much lower level of "free" endogenous formaldehyde is also much more consistent with the relative potency of exogenous vs. endogenous formaldehyde in forming tumors in the rat nose. So this analysis suggests that over 97% of the measurable formaldehyde is reversibly bound or sequestered in a way that keeps it from reacting with DNA … and causing tumors.

While the mathematical models used here are anchored in data, they are clearly extrapolations. As you are putting together your talks, any information your could provide to support, refine, or negate these predictions would be helpful!

Thanks,
-Paul

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Paul M. Schlosser
NCEA, U.S. EPA
M.D. B243-01
RTP, NC 27711
T: 919-541-4130
F: 919-685-3330
E: schlosser.paul@epa.gov
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